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Research Note

An improved DNA extraction procedure for plant tissues with a high phenolic content

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Isolation of DNA from plant tissues which have a high phenolic content is often difficult. *Tagetes minuta* L. achenes have darkly pigmented fruit walls and it is exceptionally difficult to isolate nucleic acids from them. These achenes thus served as a good model for the development of a modified DNA extraction procedure which works well for tissues with a high phenolic content.

The presence of phenolic compounds within plant tissues often results in difficulties for plant biochemists and molecular biologists. These compounds result in problems with either nucleic acid or protein extractions from such tissues as they can degrade proteins or interfere with enzymes used in subsequent manipulations of these extracts. Phenolic compounds can form hydrogen bonds with the oxygen groups involved in peptide bond formation or can covalently modify amino acid residues (Gegenheimer 1990).

Tagetes minuta L. achenes have darkly pigmented fruit walls containing large amounts of phenolic compounds. Extraction of proteins and nucleic acids from these achenes is problematic as all standard extraction procedures result in dark brown extracts which are either degraded or cannot be manipulated enzymatically. A method for extraction of proteins from *T. minuta* achenes has been published previously (Hills *et al.* 2001). This paper outlines a procedure for the isolation of DNA from these achenes, which has also been used successfully on other plant species and tissues with high phenolic content within our laboratory.

An existing CTAB (cetyltrimethylammonium bromide) DNA isolation protocol (Richards 1997) was modified by incorporating a number of additional steps to eliminate the phenolics and other contaminating compounds. Insoluble PVP (PVPP, 5% w/v) was rehydrated overnight in 2ml CTAB extraction buffer (2% w/v CTAB; 100mM Tris-Cl, pH 8.0; 20mM EDTA, pH 8.0; 1.4M NaCl) in 15ml centrifuge tubes. Immediately before use, 2% v/v 2-mercaptoethanol was added to the extraction buffer. Achenes (0.25g) were imbibed on Whatman's No. 1 filter paper discs soaked in distilled water for 24h at 25°C. The achenes were then blotted dry and ground to a fine powder in a pre-chilled mortar and pestle using liquid nitrogen. The extraction buffer containing the PVPP was pre-warmed to 65°C and the powdered achenes added to it before they could thaw. Samples were incubated at 65°C for 1h. An equal volume of 24:1 chloroform:isoamyl alcohol was then added and the contents mixed well by inversion. Tubes were centrifuged at 7 500 x *g* and 4°C for 5min. The aqueous phase was removed to a fresh tube and one tenth volume warm (65°C) CTAB/NaCl solution (10% CTAB; 0.7M NaCl) added. This was then extracted with 24:1 chloroform:isoamyl alcohol and centrifuged as before. One volume of CTAB precipitation solution (1% w/v CTAB; 50mM Tris-Cl, pH 8.0; 10mM EDTA, pH 8.0) was added to the aqueous phase in a fresh tube and the contents mixed well by inversion. The extract was then incubated at 65°C for 1h before centrifuging as before. The DNA pellet was resuspended in 500µl high salt TE buffer (10mM Tris-Cl, pH 8.0; 0.1mM EDTA, pH 8.0; 1M NaCl) and extracted with an equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol. Following centrifugation, the supernatant liquid was extracted with 24:1 chloroform:isoamyl alcohol and the phases separated by centrifugation. The aqueous phase was placed in a fresh centrifuge tube and one tenth volume 5M NaCl and three volumes ice-cold ethanol added. The DNA was allowed to precipitate overnight before being washed in 70% ethanol and resuspended in 20µl TE buffer.

The DNA pellet obtained by following the modified procedure was not brown and gelatinous as it was following isolation using the original protocol. Electrophoretic analysis on a 1.5% agarose gel showed much less degradation of the genomic DNA (Figure 1). Enzymatic manipulation such as PCR and restriction digestion was also possible with the DNA from the modified procedure, but not with the DNA from the original method (data not shown). This procedure thus

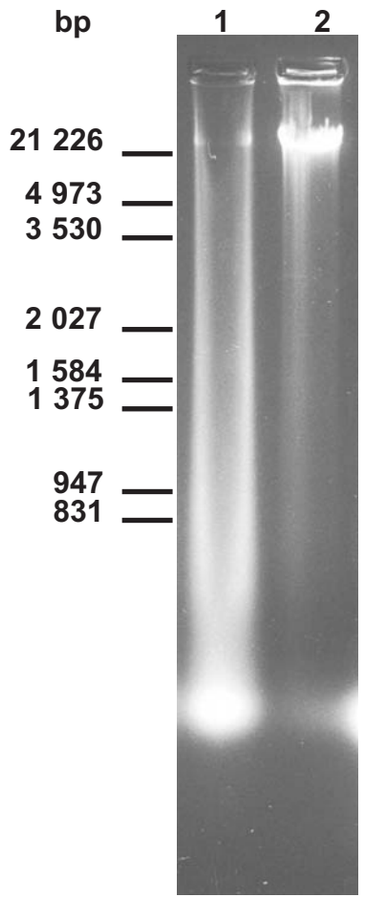

Figure 1: Comparison of genomic DNA isolated from *Tagetes minuta* achenes using a standard CTAB procedure (Richards 1997) (lane 1) and the modified procedure (lane 2). Note the greater amount of high molecular weight DNA in lane 2 and the reduced smearing as a result of decreased degradation of the DNA

represents an improvement over the standard CTAB isolation protocol and allows for the extraction of high quality DNA from tissues which contain high levels of phenolic compounds.

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